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Prevalence and Molecular Diversity of Hepatitis B Virus and Hepatitis Delta Virus in Urban and Rural Populations in Northern Gabon in Central Africa[▽]

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The prevalence of hepatitis B virus (HBV) surface antigen was significantly higher in urban (12.9%) than in rural (7.6%) populations (P = 0.003), but no difference was found in the prevalence of hepatitis delta virus (HDV), which was high in both populations. Phylogenetic analysis showed the circulation of HBV-A3 and -E genotypes and the presence of HDV-1, HDV-7, and HDV-8 clades.

Hepatitis B virus (HBV) and hepatitis delta virus (HDV) are highly endemic in Africa (1, 2); however, little information is available on the origin, circulation, and genetic diversity of these viruses in central Africa.

We reported recently that the prevalence of HBV surface antigen (HBsAg) in pregnant women in Gabon was 9.2% and that 15.6% had antibodies to HDV. Furthermore, two genotypes of HBV (subgenotype A3 and genotype E) and HDV-1 and HDV-8 clades were found in this population (7). The previous study was, however, restricted to pregnant women, and we considered that more-extensive studies of HDV clades in the general population were needed in order to characterize the circulation of these viruses in indigenous African populations. With this aim, we assessed the prevalence and genetic diversity of HBV and HDV in urban and rural populations in Gabon and compared the distributions of the HBV genotypes and HDV clades in these two areas with those of neighboring African countries.

Two epidemiological surveys were conducted in the north part of the country, in the province of Woleu-Ntem (157,013 inhabitants), which is characterized by a high population density in the cities and a very low population density in rural areas (3). The rate of immigration from Equatorial Guinea, Cameroon, and Congo is high, representing more than 22% of the total immigration rate in Gabon (9). In the first survey, 394 samples (from 203 women and 191 men) were collected in the main city of the region, Oyem. In the second survey, 961 samples (from 565 women and 396 men) were collected in 34 villages in the same province. We used the cluster sampling method and obtained ethical clearance for the study from the local public health authorities; each person gave informed consent before blood was taken.

The presence of HBsAg was assessed with the Monolisa Ag HBs-Plus test (Bio-Rad, Marnes la Coquette, France). The presence of HDV total antibodies in all HBsAg-positive samples was determined with the Murex anti-Delta (total) assay (Abbott/Murex Diagnostic Division, Wiesbaden, Germany).

Molecular and phylogenetic characterizations of HBV and HDV were performed as described previously (7). To determine the HBV genotype and HDV clade of the new Gabonese strains, we amplified and sequenced a 377-bp fragment of the *HBV-S* gene and a 326-bp fragment of the *HDV-sHD* gene. These are the fragments usually used for phylogenetic analysis and are therefore the predominant sequences in the GenBank databases. One complete HBV genome was also sequenced and characterized.

As shown in Table 1, the overall prevalence of HBsAg was significantly higher in urban (12.9%) than in rural (7.6%) areas (P = 0.003). Conversely, the prevalences of antibodies to HDV among HBsAg carriers were extremely high in both the urban and the rural areas (P not significant). Persons aged 15 to 20 years in rural areas were more frequently HBV and HDV positive than those in urban settings (P not significant), and a significant difference was found between HBsAg-positive males and females living in rural areas (P = 0.04). The prevalence of HBsAg was significantly higher (P = 0.03) among men in the urban area (16.2%) than among those in the villages (9.8%). Interestingly, only in rural areas were men more frequently HBsAg carriers (9.8%) than women (6.0%) (P = 0.04).

A 377-bp fragment of the *HBV-S* gene was obtained from 13 persons (7 males and 6 females), 9 in the urban area and 4 in the villages. The neighbor-joining tree method showed that the HBV strains belonged to subgenotype HBV-A3 and genotype HBV-E (Fig. 1A).

A 326-bp fragment of the *sHD* gene of HDV was obtained from 17 HDV-infected individuals (8 males and 9 females), 7 in the urban area and 10 in the villages. The phylogenetic analyses indicated splitting of the HDV-1 clade into two subclades, one made up of HDV strains from Canada and the Central African Republic and the other subdivided into two

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TABLE 1. Prevalence of HBV and HDV in urban and rural populations in north Gabon, by age and sex

Population and age range (yr)	No. of positive samples/no. tested (%)				Value[a] for total population					
	Male		Female							
	HBsAg	Anti-HDV	HBsAg	Anti-HDV	No. of HBsAg-positive samples/no. tested (%)	OR	95% CI	No. of anti-HDV-positive samples/no. tested (%)	OR	95% CI
Urban										
14–20	6/48 (12.5)	4 (66.7)	9/70 (12.9)	6 (66.7)	15/118 (12.7)	0.97	0.5–1.9	10/15 (66.6)	0.8	0.2–3.6
21–30	16/72 (22.2)	13 (81.3)	7/83 (8.4)	4 (57.1)	23/155 (14.8)	1.3	07–2.4	17/23 (73.9)	1.3	0.3–5.6
31–40	5/33 (15.1)	3 (60.0)	2/30 (6.6)	1 (50.0)	7/63 (11.1)	0.8	0.4–1.9	4/7 (57.1)	0.5	0.07–4.0
41–50	4/18 (22.2)	4 (100.0)	2/15 (13.3)	1 (50.0)	6/33 (18.2)	1.6	0.6–4.0	5/6 (83.3)	2.3	0.2–21.2
51–60	0/20	0	0/5	0	0/25			0/25		
Total	31/191 (16.2)	24/31 (77.4)	20/203 (9.9)	12/20 (60.0)	51/394 (12.9)			36/51 (70.6)		
Rural										
15–20	4/9 (44.4)	4 (100.0)	3/37 (8.1)	3 (100)	7/46 (15.2)	2.3	0.9–5.4	7/7 (100)		
21–30	7/39 (17.9)	6 (85.7)	8/80 (10.0)	6 (75.0)	15/119 (12.6)	1.4	0.8–2.8	12/15 (80.0)	2.8	0.7–17.0
31–40	9/65 (13.8)	7 (77.8)	12/100 (12.0)	9 (75.0)	21/165 (12.7)	2.1	1.2–3.6	16/21 (76.2)	2.3	0.7–9.4
41–50	7/60 (11.7)	4 (57.1)	7/112 (6.3)	2 (28.6)	14/172 (8.1)	1.1	0.6–2.1	6/14 (42.9)	0.4	0.09–1.4
51–60	5/93 (5.4)	2 (40.0)	2/160 (1.3)	0	7/253 (2.8)	0.3	0.1–0.6	2/7 (28.6)	0.2	0.04–1.1
>60	7/130 (5.4)	3 (42.9)	2/76 (2.6)	0	9/206 (4.4)	0.5	0.2–1.0	3/9 (33.3)	0.2	0.04–1.3
Total	39/396 (9.8)	26/39 (66.7)	34/565 (6.0)	20/34 (58.8)	73/961 (7.6)			46/73 (63.0)		

[a] OR, odds ratio; CI, confidence interval.

distinct groups (Fig. 1B). The first group contains strains from all over the world, including one rural strain from Gabon, and the second group was made up of only the Gabonese HDV strains (three urban and six rural strains). Two HDV strains originating from rural areas belonged to the HDV-7 clade, and the remaining strains from both urban and rural areas fell into the HDV-8 clade.

We present here the first analysis of the prevalences of HBV and HDV and of circulating strains in a large population study in urban and rural areas of Gabon. Previously, no significant difference in HBsAg prevalence between urban and rural populations was reported (1). In our study, the prevalence of HBsAg was significantly higher in the city than in the rural areas. This might be partly explained by the heavy concentration of the population in the main city and by extensive population movement due to trade with neighboring countries.

More men than women were infected with HBV and HDV, and a significant difference in prevalence of HBsAg was observed between urban and rural populations. These findings are in accordance with previous results, showing that chronic carriage of HBsAg in Africa is usually related to gender, with statistically significantly higher carriage rates in males than in females (4, 12). Moreover, young males (aged 14 to 20) in rural settings were more frequently HBsAg positive than females. Information collected at blood sampling indicated no difference in cultural practices (such as scarification) among males and females, but young boys moved more frequently to the city than girls. The higher rate of chronic HBV infection among males may be due either to a prolonged replicative phase of the virus in boys or to differences in sexual behavior (4).

Many factors could be responsible for the transmission of HBV and HDV, including environmental, behavioral, and cultural factors (4). Moreover, HDV infection has a variable influence on the course of hepatitis B, and the clinical severity of dual infections probably depends on aspects such as the endemicity of HDV in the area, the degree of HBV viremia, and the genotypes of HBV and HDV (2).

We confirmed previous results from our group and others, showing that the HBV-A3 subgenotype and the HBV-E genotype are present throughout central and west Africa (5, 7, 8, 10). The new HBV strains described in this study were not restricted to a particular area, as HBV-A3 and -E strains were found in both rural and urban areas.

HDV is highly endemic in central Africa, with eight described clades (6, 7, 11). The first HDV strains were isolated and characterized from Africans living in France (6, 10). We showed recently that HDV-1 and HDV-8 clades are present in pregnant women in Gabon, and we provided the first evidence that HDV-8 is indigenous to Africa (7). In the present study, a large number of sequences were obtained, showing wide genetic diversity in the HDV-1, HDV-7, and HDV-8 clades, confirming that these HDV strains are endemic in the general population of Gabon. Two new strains from Gabon within the HDV-7 clade were closely related to HDV strains from Cameroon, indicating that this clade is also endemic in the country. In the HDV-1 clade, 9 of 10 newly characterized sequences from general population samples clustered with a strain previously described by our group, originating from pregnant Gabonese women. This Gabonese HDV-1 subclade is therefore widespread in the country. More-extensive studies are needed to confirm this clustering.

In conclusion, our data provide clear evidence that HBV and HDV are highly endemic in indigenous general populations of Gabon, with wide genetic diversity.

Nucleotide sequence accession numbers. Sequences were deposited in GenBank under the following accession numbers: for the new HBV-A3 and HBV-E strains, FJ349266 to FJ349277; for the complete genome sequence obtained for the

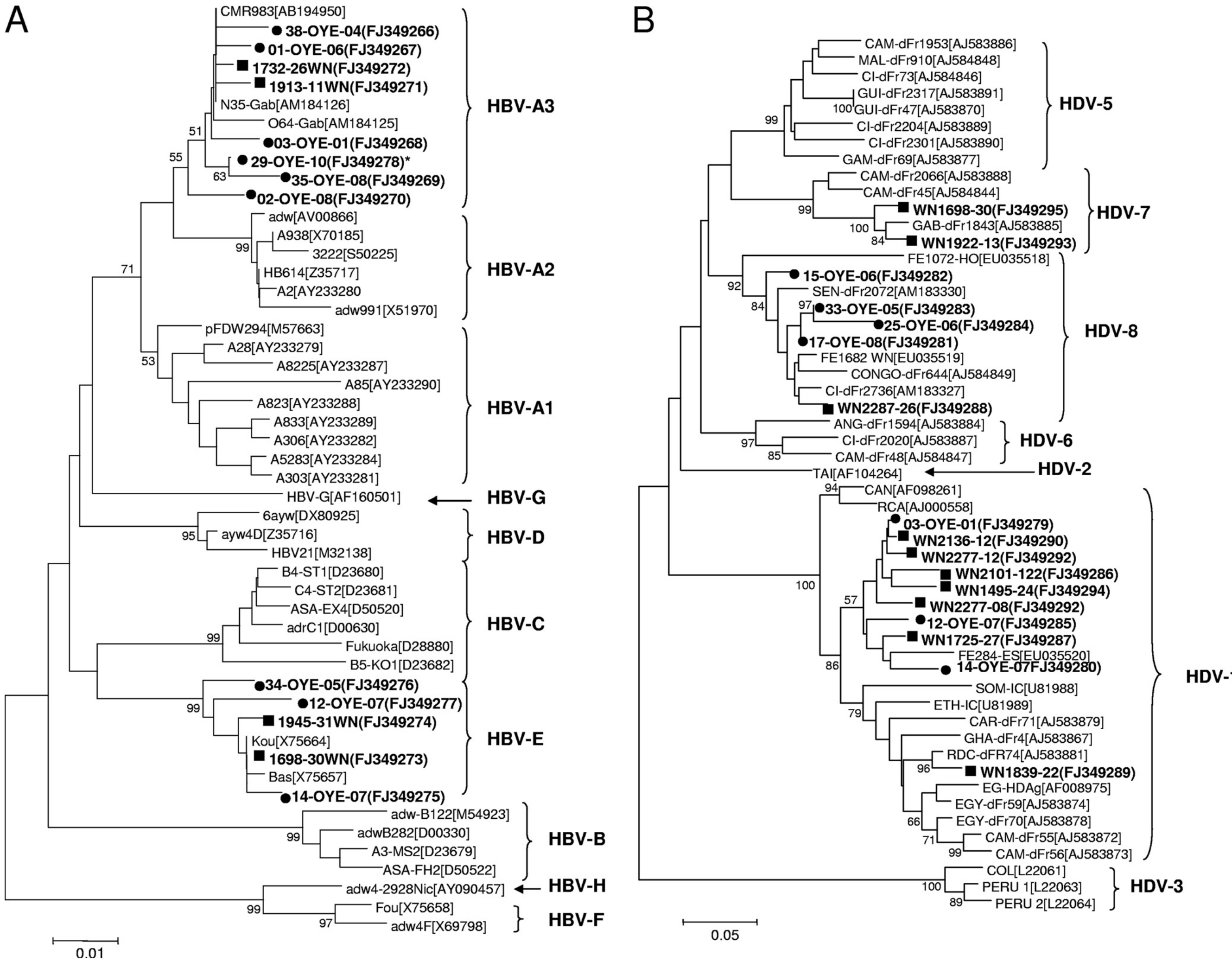

FIG. 1. Phylogenetic analysis of HBV and HDV strains obtained from rural and urban populations in northwest Gabon in central Africa. (A) Phylogenetic analysis of a 341-bp fragment of the *HBV-S* gene from different HBV isolates by the neighbor-joining method, with HBV-G (AF160501) as the outgroup. Eight HBV-A3 sequences and five HBV-E sequences (highlighted in bold) (●, obtained from the urban area; ■, obtained from the rural area) were analyzed with 37 HBV sequences from GenBank. The GenBank accession numbers for the new HBV-A3 and HBV-E strains from Gabon are FJ349266 to FJ349277. * indicates a complete genome sequence that was obtained for the 29-Oyem-10 sample (GenBank accession number FJ349296). The genome was found to be only 3,212 bp in length due to deletion of 9 nucleotides in the X gene (nt 391 to nt 399). A separate phylogenetic analysis of the partly overlapping open reading frames coding for P (polymerase-reverse transcriptase protein Pol; bootstrap value, 99%), S (envelope proteins S, M, and L; bootstrap value, 91%), C (core protein; bootstrap value, 65%), and X (transcriptional *trans*-activator protein; bootstrap value, 67%) confirmed this HBV-A3 clustering (data not shown). (B) Phylogenetic analysis of a 326-bp fragment of the HDV-sHD gene from various HDV isolates with the neighbor-joining method, with the HDV-3 clade as the outgroup. HDV sequences from Gabon (highlighted in bold) (●, urban strains; ■, rural strains) were analyzed with 36 HDV sequences from GenBank. The GenBank accession numbers for the HDV strains isolated from the general population of Gabon are FJ349279 to FJ349295.

29-Oyem-10 sample, FJ349296; and for the HDV strains isolated from the general population of Gabon, FJ349279 to FJ349295.

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